Radiolabelled monoclonal antibodies for molecular imaging of chronic inflammatory diseases

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Chapter 7

Summary

This thesis describes the synthesis and clinical application of different radiolabelled monoclonal antibodies for receptor mapping and therapy decision making in patients affetced by organ-specific autoimmune diseases.

Chapter 1 is a general introduction focusing on the pathophysiology of inflammatory processes and the radiochemistry of SPECT radiopharmaceuticals to visualize them. This chapter also reviews research performed in the past and includes an overview of the current state of nuclear medicine imaging modalities. Particular emphasis is given to radiolabelled monoclonal antibodies that could provide ideal imaging tools for the diagnosis of several inflammatory disorders. Therapy decision making is generally based on patho-physiological and patho-biochemical examinations and on clinical history of patients. Nowadays, a number of effective but expensive biological therapies using monoclonal antibodies are emerging. However, biological therapies can often fail due to the absence or low expression of therapeutic target molecules in the inflammatory lesions. Before the selection of a biological therapy, the clinician must therefore know not only whether inflammation is present and where it is located, but also which type of cells are involved and, most importantly, what type of receptors or biomarkers are present in the inflammatory lesion. Radiolabelled monoclonal antibodies are highly specific radiopharmaceuticals for their targets and a positive scintigraphic image indicates that the target molecules are present in the inflammatory lesion. This approach may also provide the rationale to select a specific therapy for the patient with an inflammatory disease or, at least, gives an explanation for the failure of any particular targeted therapy. In the subsequent chapters, the synthesis and evaluation of several radiolabelled monoclonal antibodies for imaging of diverse targets in inflammatory lesions is described.

In **Chapter 2**, the use of ^{99m}Tc-infliximab as a radiopharmaceutical to detect the presence of tumor necrosis factor α (TNFα) within the gut mucosa of patients with active Crohn's Disease (CD) is studied. CD is a chronic inflammatory bowel disease characterized by a cellular-mediated immune response driven by cytokines that are secreted mainly by T helper 1 cells (Th1). In active phases of the disease, an increased production and release of TNFα by macrophages and monocytes of the lamina propria

has been described. Therefore, the monoclonal antibody directed against TNFα, infliximab, was radiolabelled with ^{99m}Tc and successfully preclinically evaluated as a radiopharmaceutical for scintigraphic imaging. In 10 patients with active CD refractory to conventional medical therapies, ^{99m}Tc-infliximab scintigraphy was subsequently performed. These imaging studies showed that only little TNFα was present in the affected bowel of patients with active CD. Therefore, it can be concluded that the clinical benefit that patients have from infliximab therapy is unlikely to be the consequence of a local a reduction in TNFα levels. Therefore, the mechanism of action of infliximab, in therapeutic doses, requires further investigation.

In **Chapter 3**, the role of scintigraphy with ^{99m}Tc-infliximab in predicting and monitoring the response to TNFα antagonists in patients with refractory knee monoarthritis is investigated. In addition, the prognostic potential of infliximab scintigraphy for therapy decision-making in patients to be treated with the anti-TNFα agent was evaluated. We performed scintigraphy with ^{99m}Tc-infliximab and correlate our scintigraphic data with various clinical parameters (arthritis score, ESR, CRP, PCR, VAS, and ultrasonography) that were obtained both at baseline and after the initiation of therapy. This study demonstrates that radiolabelled anti-TNFα scintigraphy could be useful for improving the selection of those patients who could benefit most from local or systemic therapy with TNFα antagonists and for obtaining an objective evaluation of therapy efficacy, also in asymptomatic patients. However, a large prospective study is still required to determine the predictive value of this technique.

In **Chapter 4**, a radiolabelled humanized anti-CD3 monoclonal antibody (visilizumab) for imaging human T-lymphocytes was investigated. We hypothesised that the use of a radiolabelled anti-CD3 antibody might serve as a diagnostic tool for imaging T-cell trafficking and lymphocytic infiltration of tissues and organs affected by organ-specific autoimmune diseases. Visilizumab was labelled with ^{99m}Tc with high labelling efficiency and high specific activity using the bispecific chelator HYNIC. After the labelling procedure, biochemical integrity and *in-vitro* binding activity to CD3 positive cells were retained. *In-vivo* targeting experiments showed a proportional increase of specific uptake with the number of injected cells, both at 6 and 24 h. An *in-vivo*

competition study demonstrated a more than 60% decrease in uptake after blocking of the CD3 epitope with an excess of unlabelled antibody. In SCID mice, hPBMCs migration to different tissues was detected by ^{99m}Tc-labelled visilizumab and confirmed by histology. Our results demonstrated that this radiolabelled monoclonal antibody could be a valuable tool for the study of human T lymphocyte trafficking and lymphocytic infiltration of tissues and organs.

In **Chapter 5**, our results of scintigraphy with radiolabelled rituximab (anti-CD20 monoclonal antibody) in patients with chronic autoimmune diseases are described. CD20 positive B cells have been found in pathological infiltrates in tissues affected by autoimmune diseases and are implicated in disease progression. We hypothesized that a radiolabelled anti-CD20 antibody could be used as a prognostic tool for therapy decision-making. Scintigraphy with ^{99m}Tc-rituximab in patients with autoimmune diseases showed rapid and persistent spleen uptake, and kidneys appeared to be the prominent route for excretion of the radioactivity. Inflamed joints and salivary glands were clearly detectable at 6h p.i. in patients with arthritis and Sjogren's syndrome, but muscle and skin uptake in patients with Dermatomyositis was more difficult to detect. In this study, we concluded that ^{99m}Tc-labelled rituximab can be used for imaging B-lymphocyte infiltration, thus providing a rationale for anti-CD20 antibody therapy.

In **Chapter 6**, the radiolabelling and *in vitro* and *in vivo* studies of a new humanised anti-HLA-DR monoclonal antibody (1D09C3) are described. It is known that HLA-DR is highly expressed on B-cell lymphomas and in a variety of autoimmune and inflammatory diseases. Recent studies showed that HLA-DR protein status predicts survival in patients with B-cell lymphomas, but it is not yet known whether it is possible to obtain this information *in-vivo* by non-invasive imaging modalities. Therefore, a radiolabelled fully humanised IgG4 monoclonal antibody can provide a useful tool for molecular imaging and therapy decision making. Anti-HLA-DR monoclonal antibody was labelled with the highest labelling efficiency and specific activity using the direct 2-mercaptoethanol reduction method. In vitro analysis demonstrated that the stability, structural integrity and specific binding properties of the monoclonal antibody were retained after labelling. Animal experiments confirmed

the high binding specificity of the radiolabelled monoclonal antibody to the HLA-DR positive cells in vivo. In conclusion, our data suggest that this radiolabelled monoclonal antibody shows favorable properties for scintigraphic imaging and therefore deserves to be investigated as a prognostic and diagnostic tool in lymphoma patients and in patients with autoimmune chronic inflammatory diseases.

Thus, several monoclonal antibodies directed against a variety of targets involved in inflammatory diseases have been radiolabelled. Evaluation of these radiolabelled monoclonal antibodies in preclinical studies and small proof-of-concept studies in patients showed highly promising results. Although these results need to be confirmed in larger prospective studies, the preliminary data indicate that this new molecular approach to disease diagnosis and therapy decision making could have revolutionary impact on the management of patients with chronic inflammatory diseases.